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Early Growth Response Factor-1 Is Associated With Intraluminal Thrombus Formation in Human Abdominal Aortic Aneurysm

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Objectives The goal of this study was to investigate the expression of early growth response-1 (Egr-1), a vascular pathogenic transcription factor, and its potential relationship with tissue factor (TF), a key player during the thrombus formation in the abdominal aortic aneurysm (AAA) wall.

Background Although intraluminal thrombus is a common finding in human AAA, the molecular mechanism of the thrombus formation has not been studied.

Methods During the elective AAA repair, specimens were taken from the thrombus-covered and thrombus-free portions of the aneurysmal wall in each of 16 patients with AAA and analyzed to assess the differential expression of Egr-1 and TF. The proinflammatory and prothrombogenic activities of Egr-1 in vasculature were evaluated in vitro and in vivo by overexpressing it using adenovirus.

Results The expression of both Egr-1 and TF was significantly increased in the thrombus-covered wall compared with the thrombus-free wall, in which their up-regulation in the thrombus-covered wall was strongly correlated with each other ($p < 0.005$, $r = 0.717$). Adenoviral overexpression of Egr-1 in human vascular smooth muscle and endothelial cells was found to up-regulate the expression of TF and inflammation-related genes. Moreover, Egr-1 overexpression in endothelial cells increased their adhesiveness to monocytes and also substantially promoted the intravascular thrombus formation in vivo, as shown in the inferior vena cava ligation experiment of the rat.

Conclusions The present study demonstrates the differential up-regulation of Egr-1 in the thrombus-covered wall of human AAA and also suggests its possible contribution to the thrombogenic and inflammatory pathogenesis in human AAA. (J Am Coll Cardiol 2009;53:792–9) © 2009 by the American College of Cardiology Foundation

An abdominal aortic aneurysm (AAA) is a potentially lethal, age-related transmural degenerative disease of the distal abdominal aorta. The pathogenesis of an AAA is complex and multifactorial (1). Histologically, an AAA is characterized by mural inflammatory cell infiltration, destruction of elastin and collagen in the media and adventitia, and smooth muscle cell loss with thinning of the medial wall. These cellular and extracellular events in the aortic wall lead to progressive aortic enlargement and may lead to eventual rupture. In particular, the rupture of an AAA, the most devastating complication, has been reported to frequently occur at thrombus-covered walls, rather than at thrombus-free walls, which indicates that the presence of thrombus might predispose to the AAA rupture (2).

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Moreover, it has been suggested that the growth of thrombus may be a better predictor of aneurysmal rupture than diameter in patients with AAA. Interestingly, previous clinical studies analyzing the size and morphology of intraluminal thrombus in AAA have shown that intraluminal thrombus is frequently found at AAA in an eccentric manner but not a concentric manner (2,3). Most aneurysms

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contain thrombus, but the exact mechanisms regarding how the intraluminal thrombus occurs at the specific aortic region in aneurysm remain unresolved.

Among many molecules involved in the intraluminal thrombus formation, tissue factor (TF) is best known as a crucial prothrombotic player to initiate the intravascular coagulation cascade. A recent publication reported that the vascular wall is the important source of thrombogenic TF that drives occlusive thrombus formation after injury to medium-sized and large blood vessels (4). Although it is controversial whether TF associated with the vessel wall or derived from leukocytes plays a dominant role in the thrombus formation, it is generally accepted that TF present in the vascular wall substantially contributes to the thrombus propagation as well as the initial thrombus formation (5,6). Induction of TF in vascular walls is regulated by many factors including platelet-derived growth factor, tumor necrosis factor, and others. In particular, early growth response factor-1 (Egr-1) was recently characterized to enhance the transcription and activity of TF upon stimulation of shear stress or platelet-derived growth factor (7). In addition, Egr-1 has been implicated as a master transcription factor modulating many cardiovascular pathological processes, in that it drives the expression of diverse proadhesive, proinflammatory, and prothrombotic genes upon vascular injury (8–12).

Considering the histopathological properties of AAA, these findings lead us to speculate that the differential up-regulation of Egr-1 and subsequent overexpression of TF at a certain aneurysmal wall might be related to the thrombus formation in AAA. Therefore, the present study was intended to analyze the correlation between the expression of Egr-1 and TF at thrombus-covered and thrombus-free AAA tissue specimens in AAA and further characterize molecular functions of Egr-1 in vascular smooth muscle and endothelial cells.

Abbreviations and Acronyms

AAA = abdominal aortic aneurysm
Egr = early growth response factor
HASMC = human aortic smooth muscle cell
HUVEC = human umbilical vein endothelial cell
ICAM = intercellular adhesion molecule
IVC = inferior vena cava
MCP = monocyte chemotactic protein
MIP = macrophage inflammatory protein
PBS = phosphate-buffered saline
RT-PCR = reverse transcriptase-polymerase chain reaction
TF = tissue factor

Methods

Patient material and collection of samples. Informed consent was obtained from the time of tissue collection in accordance with protocol approved by the Institutional Review Board of Samsung Medical Center. Tissue samples were obtained from 16 selected patients, 15 men and 1 woman with a mean age of 68.6 years, undergoing elective open AAA repair. Pre-operative computed tomography scans demonstrated intraluminal thrombus-covered and thrombus-free aneurysmal walls. Aneurysmal wall was taken for the tissue specimens of thrombus-covered and thrombus-free walls, where two 2 × 3-cm tissue sections were transversely cut from thrombus-covered wall and thrombus-free aneurysmal wall, respectively. The thrombus was removed from the aortic wall. The sections free of thrombus, excess perivascular fat, and debris were fixed in formalin for immunohistochemistry or immediately snap-frozen in liquid nitrogen for ribonucleic acid (RNA) preparation.

RNA extraction and reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA (1 μg) was ex-

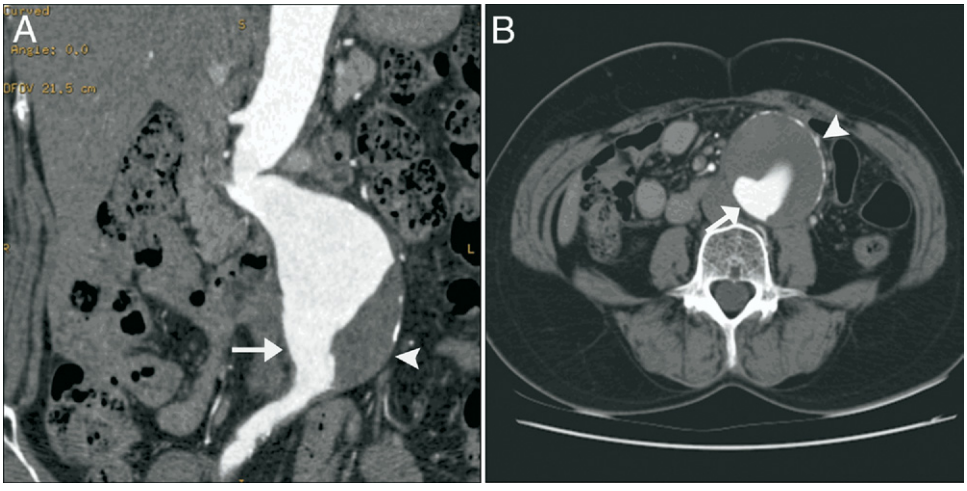

Figure 1 Abdominal CT Scans Demonstrating the Location of Intraluminal Thrombus at More Expanded AAA

(A) Coronal and **(B)** axial images of human abdominal aortic aneurysm (AAA). Tissue specimens were taken from the thrombus-covered **(arrowheads)** and thrombus-free walls **(arrows)** of each patient. CT = computed tomography.

tracted from frozen aortic wall using Trizol reagent (Invitrogen, Carlsbad, California) and was processed for complementary deoxyribonucleic acid (cDNA) synthesis using the Superscript first-strand synthesis system (Invitrogen). The cDNA was amplified with 30 to 35 cycles of polymerase chain reaction (PCR) using a primer specific for each gene. Primers were used as follows: Egr-1, forward 5′-CTGCGACATC TGTGGAAGAA-3′, reverse 5′-TGTCCTGGGAGAAA-AGGTTG-3′; TF, forward 5′-TGAAGGATGTGAAGC-AGACG-3′, reverse 5′-GCCAGGATGATGACAAGG-AT-3′; intercellular adhesion molecule-1 (ICAM-1), forward 5′-CGCTGAGCTCCTCTGCTACT-3′, reverse 5′-GT-TCTCAAACAGCTCCAGCC-3′; monocyte chemotactic protein-1 (MCP-1), forward 5′-CCCCAGTCACCTGC-TGTTAT-3′, reverse 5′-CAAAACATCCCAGGGGT-AGA-3′; macrophage inflammatory protein-1β (MIP-1β), forward 5′-TGCGTGACTGTCCTGTCTCT-3′, reverse 5′-GACAGTGGACCATCCCCATA-3′; glyceraldehydes-3-phosphate dehydrogenase, forward 5′-CGTGGAAGG-ACTCATGAC-3′, reverse 5′-CAAATTCGTTGTCA-TACCAG-3′. Each gene's expression was analyzed in triplicate at least.

Quantitative real-time PCR. Real-time PCR was carried out using the ABI PRISM 7000 (Applied Biosystems, Foster City, California) according to the manufacturer's instructions. Reactions were performed in a 20-μl volume containing cDNA, corresponding primers, and probes from Applied Biosystems. To confirm the amplification specificity, the PCR product from each primer pair was subjected to a standard curve analysis and normalized by calculating the ratio against glyceraldehydes-3-phosphate dehydrogenase expression. All reactions were performed in triplicate.

Immunohistochemistry. For the assessment of the expression of Egr-1 and TF, sections embedded in paraffin were treated with 0.3% hydrogen peroxidase. After retrieving epitopes by heating in Target Retrieval Solution (Dako-Cytomation, Carpentaria, California), sections were blocked and incubated with anti-Egr-1 immunoglobulin (Ig)G (Santa Cruz Biotechnology Inc., Santa Cruz, California), anti-TF IgG (American Diagnostica Inc., Stamford, Connecticut), or irrelevant IgG control. Then, sections were incubated with biotinylated secondary IgG (Jackson Immuno Research Laboratories Inc., West Grove, Pennsylvania) for 30 min and washed with phosphate-buffered saline (PBS), after which slides were visualized by using an avidin biotin complex-peroxidase kit (Elite kit, Vector Laboratories, Burlingame, California) and 3,3-diaminobenzidine tetrahydrochloride (Vector Laboratories). Sections were then counterstained with hematoxylin before mounting.

Cell culture and adenovirus infection. Human umbilical vein endothelial cells (HUVECs) and human aortic smooth muscle cells (HASMCs) were purchased from ScienCell (ScienCell Research Laboratories, San Diego, California). HUVECs and HASMCs were cultured in endothelial cell growth factor medium-2 (EGM-2, Clonetics, San Diego, California) and smooth muscle growth medium-2 (SMGM, ScienCell), respectively. U937 monocytes (American Type Culture Collection, Manassas, Virginia) were incubated in RPMI 1640 medium (Clonetics) with 10% fetal bovine serum. For adenoviral infection, cells at passage 6 to 9 were

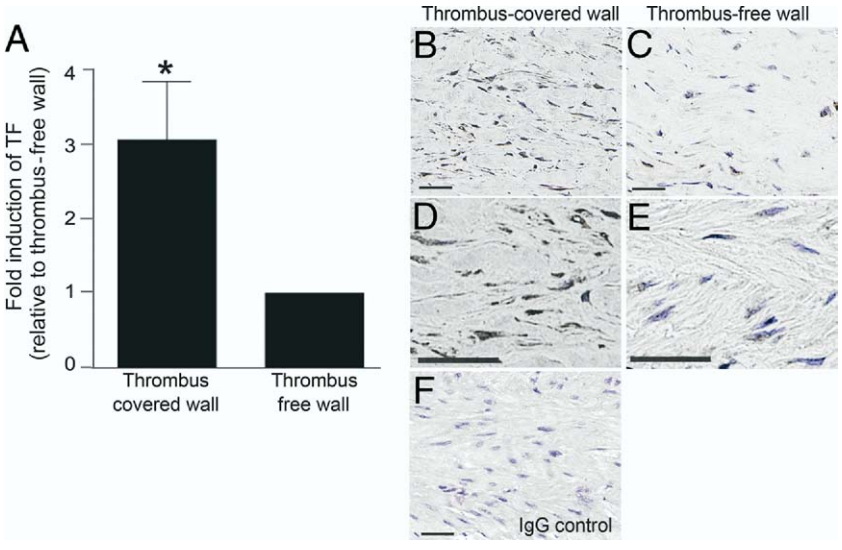

Figure 2 TF Is Highly Expressed in the Thrombus-Covered Walls But Not in the Thrombus-Free Walls in Human AAA

(A) Real-time reverse transcriptase-polymeric chain reaction shows that the expression level of tissue factor (TF) in the thrombus-covered wall was approximately 3-fold higher than that in thrombus-free walls (*$p < 0.05$ vs. thrombus-free wall). **(B to F)** Representative immunohistochemical images showing the TF expression in human abdominal aortic aneurysmal (AAA) wall. Images with irrelevant primary immunoglobulin (Ig)G **(B)**; images with thrombus-covered walls **(C and E)** and thrombus-free walls **(D and F)** in AAA stained with anti-TF IgG. Scale bar is 50 μm.

starved overnight in serum-free media and then incubated with adenoviral Egr-1 (Ad-Egr-1) or LacZ (Ad-LacZ) at 200 multiplicity of infection (MOI) for 4 h. After washing with PBS, cells were cultured in serum-free media and used for the further experiments. The method used for preparing the recombinant adenovirus was previously described in detail (13).

Monocyte adhesion assay. Ad-LacZ– or Ad-Egr-1–infected HUVECs (confluently seeded on gelatin-coated 24-well plate; 7×10^4/well) were incubated with 1×10^5 of DiI (Invitrogen)-labeled U937 cells at 37°C for 24 h. After several washings with PBS, adherent U937 cells were counted in 5 random microscopic fields (200×) by blinded investigators. These experiments were performed in triplicate at least.

TF activity. TF activity in Ad-LacZ– or Ad-Egr-1–infected HUVECs or HASMCs was measured by Actichrome TF activity kit (American Diagnostica Inc.) according to the manufacturer's instructions. In brief, cell lysates were mixed with human factor VIIa and human factor X. During incubation at 37°C, TF from cell lysates form complexes with factor VIIa, which then convert the factor X into factor Xa. The amount of factor Xa generated was measured by its ability to cleave the highly specific chromogenic substrate, releasing a paranitroanilin-chromophore with a specific change of absorption at 405 nm. TF activity was expressed in arbitrary units by reference to a standard curve constructed from recombinant human-lipidated TF. These experiments were performed in triplicate at least.

Mouse inferior vena cava (IVC) thrombosis model. IVC thrombosis was produced in C57BL/6 mice (Charles River Laboratories, Yokohama, Japan) by standardized IVC ligation with minor modification as described in the following text. Mice weighing 20 to 25 g were anesthetized by intraperitoneal injection of 80 μl of solution containing 2.215-mg ketamine (Ketara 50 mg/ml, Yuhan Co., Seoul, Korea) and 0.175-mg xylazine (Rompun 23.32 mg/ml, Bayer, Seoul, Korea) during the surgical procedure (14). After midline laparotomy, the small bowel was exteriorized from the body cavity and the IVC was directly approached and ligated with 6-0 prolene (Ethicon, Inc., Somerville, New Jersey) just below the renal veins, with another ligation just above the iliac bifurcation. Then, 2×10^8 pfu of Ad-LacZ or Ad-Egr-1 in 50 μl of normal saline was injected into the vein ligated between the renal vein and iliac bifurcation. After 30-min incubation with adenovirus, the vein ligated at proximal iliac bifurcation was untied. Two days later, mice were sacrificed and immediately snap-frozen by immersion in liquid nitrogen or stored at −70°C for further analysis. For the visualization of the IVC occlusive thrombus, mice were injected with barium sulfate (BarytgenSol, Fushimi, Tokushima, Japan) as a contrast material and their X-ray images of IVC occlusive thrombus were obtained using C-arm X-ray (VISIPLEX, CCM 620, Geilenkirchen, Germany). All procedures were performed in accordance with the guidelines for the care and use of laboratory animals published by the U.S. National Institutes of Health (NIH publication no. 85-23, revised 1996).

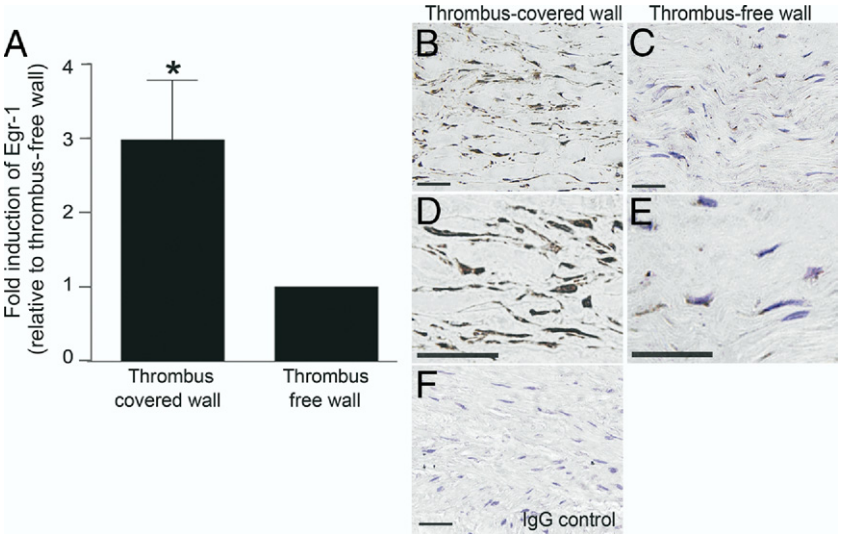

Figure 3 **Egr-1 Is Highly Expressed in Thrombus-Covered Walls, But Not in Thrombus-Free Walls in Human AAA**

(A) Real-time reverse transcriptase-polymeric chain reaction shows that the expression level of early growth response factor-1 (Egr-1) in the thrombus-covered wall was approximately 3-fold higher than that in thrombus-free walls (*$p < 0.05$ vs. thrombus-free wall). **(B to F)** Representative immunohistochemical images reveal the Egr-1 expression in human AAA wall. Images with thrombus-covered walls **(B and D)** and thrombus-free walls **(C and E)** in AAA stained with anti-Egr-1 IgG; image with irrelevant primary IgG control **(F)**. Egr-1 was highly expressed in vascular smooth muscle cells and inflammatory cells infiltrated at the media of the thrombus-covered wall, whereas Egr-1 was slightly stained in the thrombus-free wall. Scale bar is 50 μm. Abbreviations as in Figure 2.

Thrombus weight. At sacrifice, the IVC to iliac bifurcation was removed and measured for length. Then, all thrombi harvested from IVC were weighed and normalized to the length of vessel (weight/length measurement, mg/mm) (15,16).

Statistical analysis. Data are expressed as mean ± standard error of the mean. Statistical significance was evaluated using a paired *t* test or 1-way analysis of variance followed by Bonferroni's post hoc multiple comparison test, depending on the number of experimental groups. Probability values below 0.05 were regarded as statistically significant. The association between the induction fold of Egr-1 mRNA levels and that of TF mRNA levels was investigated with Spearman rank correlations.

Results

TF is up-regulated in the thrombus-covered wall of human AAA. The expression pattern of TF was examined on tissue specimens taken from the thrombus-covered and the thrombus-free walls of 16 AAA patients (Fig. 1). As shown in Figure 2A, the mRNA level of TF, quantified using quantitative real-time PCR, was significantly (approximately 3-fold) increased in the thrombus-covered walls compared with thrombus-free walls. Immunohistochemically, TF was found to be highly expressed in vascular smooth muscle cells and inflammatory cells infiltrated in the media of thrombus-covered walls, whereas little TF was expressed in the intima or media of thrombus-free normal arteries (Figs. 2B to 2E).

Egr-1 is highly up-regulated in the thrombus-covered wall of human AAA with positive correlation with TF up-regulation. To determine the association of Egr-1 with the eccentric thrombus formation in aneurysmal aortic walls, we also examined its expression pattern in aneurysmal aortic walls from patients with AAA. Consistent with the observation regarding the expression pattern of TF in AAA, the Egr-1 mRNA was also found to be significantly more abundant in the thrombus-covered walls than in the thrombus-free walls (Fig. 3A). The immunohistochemical result as shown in Figures 3B to 3E showed that the expression of Egr-1 was highly localized at vascular smooth muscle cells and infiltrated inflammatory cells at the media of the thrombus-covered walls. Of note, the induction (fold increase in a thrombus-covered wall compared with a thrombus-free wall) of Egr-1 was found to significantly correlate with the induction of TF, as shown in Figure 4 ($p = 0.0018$; $r = 0.717$). This result indicates that differential expression of Egr-1 in AAA may be associated with the eccentric thrombus formation via modulating the expression of TF, one of Egr-1 downstream target genes.

Egr-1 promotes proinflammatory and prothrombogenic activity of HASMCs and HUVECs in vitro. To investigate the role of Egr-1 overexpressed in thrombus-covered walls of AAA, vascular cells (HASMCs and HUVECs) were transfected with the adenovirus encoding human Egr-1 or LacZ. The adenoviral infection at 200 MOI for 4 h yielded the substantially high expression of Egr-1 in both cell lines, but did not induce the adenovirus-induced cellular apoptosis. Then, RT-PCR was carried out to investigate the gene expression profile in the Egr-1 overexpressed HASMCs and HUVECs, where proinflammatory genes important for the initiation and propagation of thrombus formation were carefully analyzed compared with Ad-LacZ–transfected or PBS-untreated controls. As shown in Figure 5A, adhesion molecule (ICAM-1) and inflammatory chemokines (MCP-1, MIP-1β) were significantly up-regulated in Ad-Egr-1–transfected HASMCs and HUVECs, which, however, were barely expressed in untreated and Ad-LacZ–transfected cells (Fig. 5A). This gene expression profile in Egr-1 overexpressed HUVECs might account for the monocyte adhesion data, where adhesion of human monocytic U937 cells was significantly enhanced on the Egr-1–overexpressed HUVEC monolayer compared with Ad-LacZ–transfected or untreated controls (Fig. 5B). In addition to inflammation-related genes, TF, an initiator of clotting cascade, was significantly up-regulated in both HUVECs and HASMCs after Ad-Egr-1 transfection (Fig. 5A). When mean TF activity was measured with an amidolytic assay that measures factor Xa generation, it was significantly promoted in both HASMCs and HUVECs by adenovirus-mediated overexpression of Egr-1 compared with that in other controls. These results indicate that Egr-1 might play a crucial role in regulating proinflammatory and prothrombic activities of vasculature by modulating several

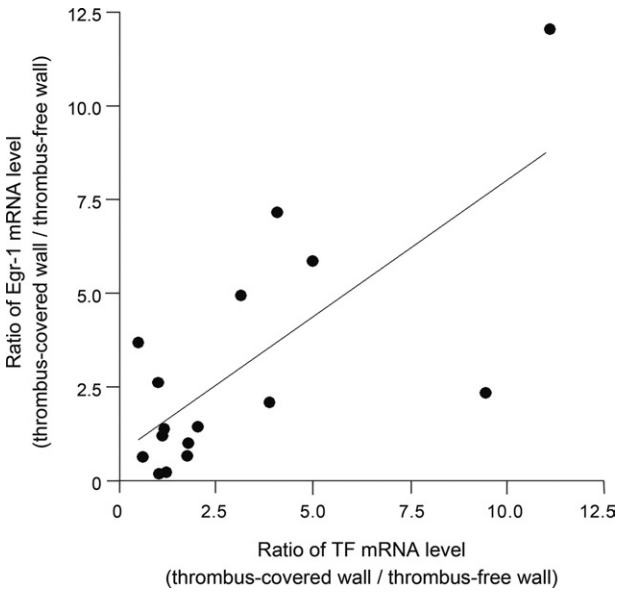

Figure 4 The Induction of Egr-1 Positively Correlated With That of TF

The significant positive correlation between the induction fold of Egr-1 and TF (ratio of their expression in a thrombus-covered wall relative to that in a thrombus-free wall). $p = 0.0018$; $r = 0.717$, $n = 16$. mRNA = messenger ribonucleic acid; other abbreviations as in Figure 1.

relevant genes, including TF, adhesion molecules, and chemotactic cytokines.

Egr-1 increases thrombus formation in a mouse IVC model. To investigate whether or not the vascular up-regulation of Egr-1 indeed caused the thrombus formation, the IVC in mouse was ligated and treated with saline, Ad-LacZ, or Ad-Egr-1. During this experiment, we observed that the thrombus formation occurred more than 90% of the time in saline-, Ad-LacZ–, and Ad-Egr-1–treated groups, whereas it happened at a significantly lower frequency in sham controls. In Figure 6A, post-mortem angiographic images obtained from each group showed that the thrombus formation was substantially promoted in the Ad-Egr-1–transfected IVC compared with other control groups. This result was consistent with the hematoxylin and eosin-stained images of the IVC from each group, where severe occlusive thrombosis was frequently observed in the IVC transfected with Ad-Egr-1 (Fig. 6B). Because the thrombus size has been considered a reproducible assessment of thrombus formation, the thrombus formation was quantitatively evaluated by measuring the weight of IVC-associated thrombus. As shown in Figure 6C, the thrombus size was found to significantly increase in the IVC from Ad-Egr-1–transfected mice compared with those from Ad-LacZ–transfected or saline controls (1.7- and 1.94-fold increase in thrombus weight), which was in accordance with the angiographic and hematoxylin and eosin-stained images of IVC tissues. The adenovirus-mediated overexpression of LacZ or Egr-1 in the IVC vein wall was confirmed by X-gal staining (data not shown) or RT-PCR (Fig. 6D).

Discussion

The main findings of the present study are as follows: 1) TF was predominantly up-regulated in the thrombus-covered wall compared with thrombus-free wall in human AAA; 2) Egr-1 was also significantly up-regulated in the thrombus-covered wall of human AAA with a positive correlation with TF induction; and 3) Egr-1 overexpression in vascular cells promoted the inflammatory reaction and significantly contributed to the thrombus formation.

The significance of Egr-1 induction in the thrombus-covered wall of an AAA could give insight into the molecular events programming thrombus formation in human AAA, because Egr-1 is a potent transcriptional factor that regulates the expression of divergent gene families, in particular, those related with a cascade of thrombotic events. Among a plethora of genes whose induction is triggered by Egr-1, TF, barely expressed in the normal vascular wall, was found to be substantially overexpressed in the thrombus-

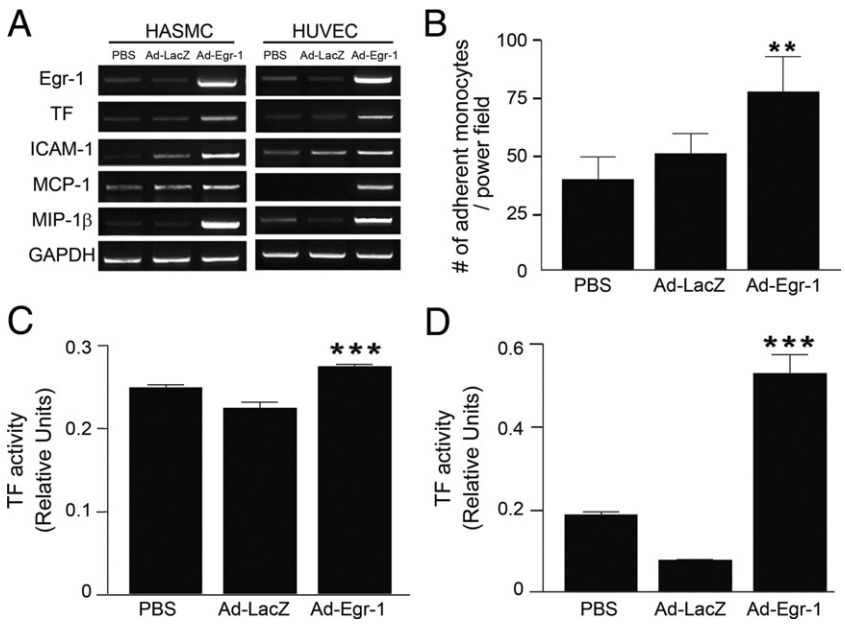

Figure 5 Egr-1 Promotes Proinflammatory and Prothrombogenic Activity of HASMC and HUVEC In Vitro

(A) TF and inflammation-related genes were significantly up-regulated in adenoviral Egr-1–transfected human aortic smooth muscle cells (HASMCs) and human umbilical vein endothelial cells (HUVECs). After infection with adenoviral (Ad)-Egr-1 or β-galactosidase (LacZ) at 200 multiplicity of infection (MOI) for 4 h, cells were cultured in serum-free media and prepared for the reverse transcriptase-polymeric chain reaction. Glyceraldehydes-3-phosphate dehydrogenase (GAPDH) served as a loading control. Similar results were obtained in triplicate experiments. **(B)** Egr-1 increased the adhesiveness of HUVECs to human monocytes (U937). The number of adherent monocytes on HUVECs was quantified and compared with other groups (**$p < 0.01$ vs. Ad-LacZ). **(C and D)** Egr-1 enhanced the TF activity in HASMCs and HUVECs. A chromogenic TF assay shows the increase of factor Xa generation in Ad-Egr-1–transfected HASMCs and HUVECs. ***$p < 0.001$ versus Ad-LacZ, phosphate-buffered saline (PBS). ICAM-1 = intercellular adhesion molecule-1; MCP-1 = monocyte chemotactic protein-1; MIP-1β = macrophage inflammatory protein-1β; other abbreviations as in Figure 1.

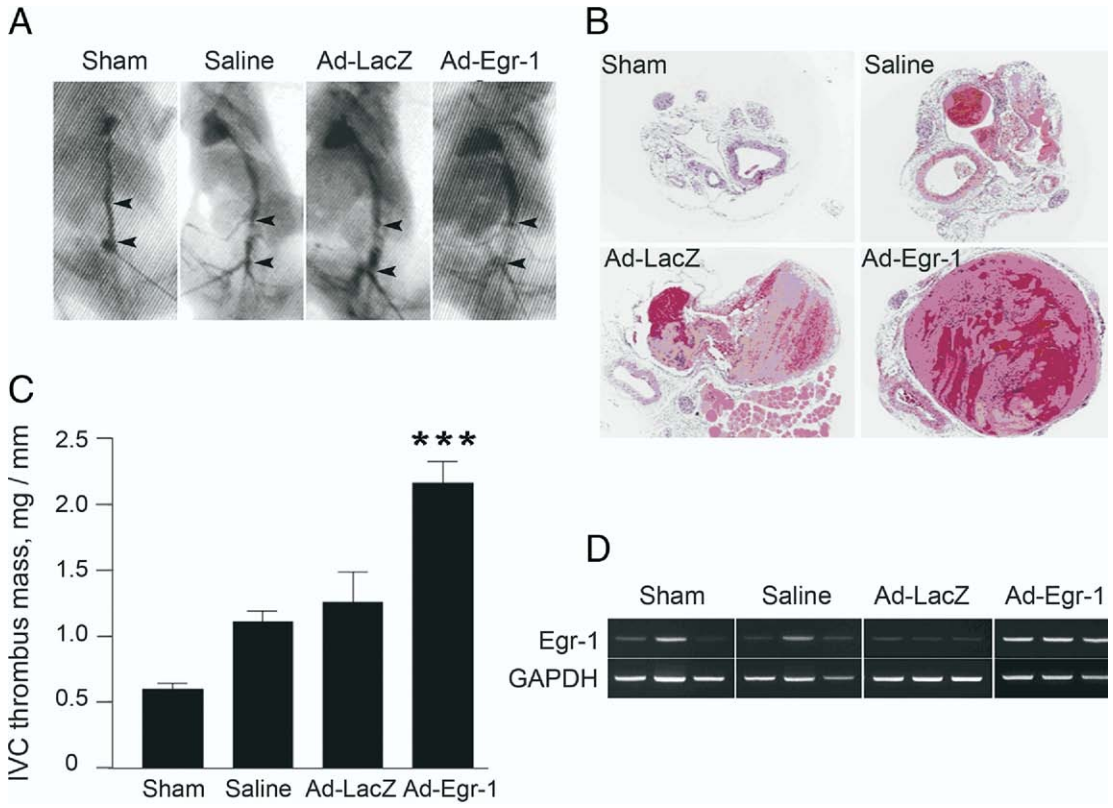

Figure 6 **Egr-1 Promotes the Intravascular Thrombus Formation in a Mouse IVC Experiment**

Representative computed tomography **(A)** and hematoxylin and eosin-stained **(B)** images of the inferior vena cava (IVC) in mice that were treated with saline, Ad-LacZ, or Ad-early growth response factor-1 (Egr-1) after IVC ligation or sham operation. **Arrowheads** indicate the ligation site. **(C)** The thrombus mass was increased in the IVC from Ad-Egr-1–transfected mice compared with that seen in the Ad-LacZ–transfected or saline-treated mice (***$p < 0.001$ vs. Ad-LacZ). **(D)** The adenovirus-mediated overexpression of Egr-1 in the IVC vein wall was confirmed by reverse transcriptase-polymerase chain reaction. Abbreviations as in Figure 5.

covered vascular wall of an AAA compared with the thrombus-free wall. Indeed, the IVC ligation experiment demonstrated that Egr-1 overexpression in vascular walls was sufficient to generate the intravascular thrombus formation. In this regard, the significant overexpression of Egr-1 and its correlation with TF induction in a thrombus-covered wall of an AAA implies that Egr-1 might be closely associated with the thrombus formation in human AAA.

In addition to the expression of TF, Egr-1 was shown to up-regulate a variety of inflammation-related genes such as ICAM-1, MCP-1, and MIP-1β in vascular endothelial and smooth muscle cells, which might facilitate the recruitment and subsequent infiltration of macrophage and leukocytes into an Egr-1–overexpressed vascular site in human AAA. Moreover, these inflammatory cells are known to release a cascade of cytokines that induce the expression and activation of many proteases such as matrix metalloproteases (17). When considering the severe inflammation and frequent rupture of AAA in the thrombus-covered wall, the significant up-regulation of Egr-1 in the thrombus-covered wall might be not only involved with the initial intra-arterial thrombus formation, but also associated with pathophysiological features of AAA. However, the issue of whether the observed high induction of Egr-1 in the thrombus-covered wall of an AAA is a causative factor in aneurysm development or simply a consequence of the disease process requires further investigation.

According to biomechanical flow studies using asymmetric AAA models and the flow Doppler study with AAA patients, both laminar and turbulent flows are present and induce dynamic fluid shear stresses through aneurysmal walls. In particular, it has been found that a laminar flow parallel to the wall induces a relatively minor impact on the aneurysmal wall, whereas a turbulent flow causes a pronounced wall shear stress, presumably being responsible for aortic enlargement and rupture (18–20). In numerical simulation models of AAA, turbulent flows were shown to produce the maximum shear stress at a distal end of aneurismal bulge, being assumed to cause endothelial damages and precipitate thrombus formation. Considering that Egr-1 is one of the genes whose expression and activity are modulated by fluid shear stress, it is likely that Egr-1 is selectively up-regulated and then stimulates the TF expression at a region with high shear stress (i.e., the distal end of

a highly dilated aneurysmal wall, where intraluminal thrombi were indeed frequently found). However, it is also possible that thrombus formation might precede the Egr-1 up-regulation, in particular at the most dilated aneurysmal wall, in that a recirculating blood flow under low shear stress at this region predisposes to platelet deposition leading to arterial thrombogenesis, and platelet-derived growth factor secreted from accumulated platelets is one of the known Egr-1 inducers (21).

Although further studies are necessary to define the role of Egr-1 as a primary force or an epiphenomenon in the initiation and progression of human AAA, the present study demonstrates that the thrombus formation in human AAA could be related to the differential expression of Egr-1. We showed that Egr-1 induced proadhesive, proinflammatory, and prothrombogenic properties in vascular walls, demonstrating a feasible active role of Egr-1 in the thrombus formation and progression of AAA. These results might provide some clues to understanding the complex and multifactorial mechanisms involved in the pathogenesis of AAA.

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